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Indices Identifying Subsurface Microbial Communities That Are Adapted to Organic Pollution

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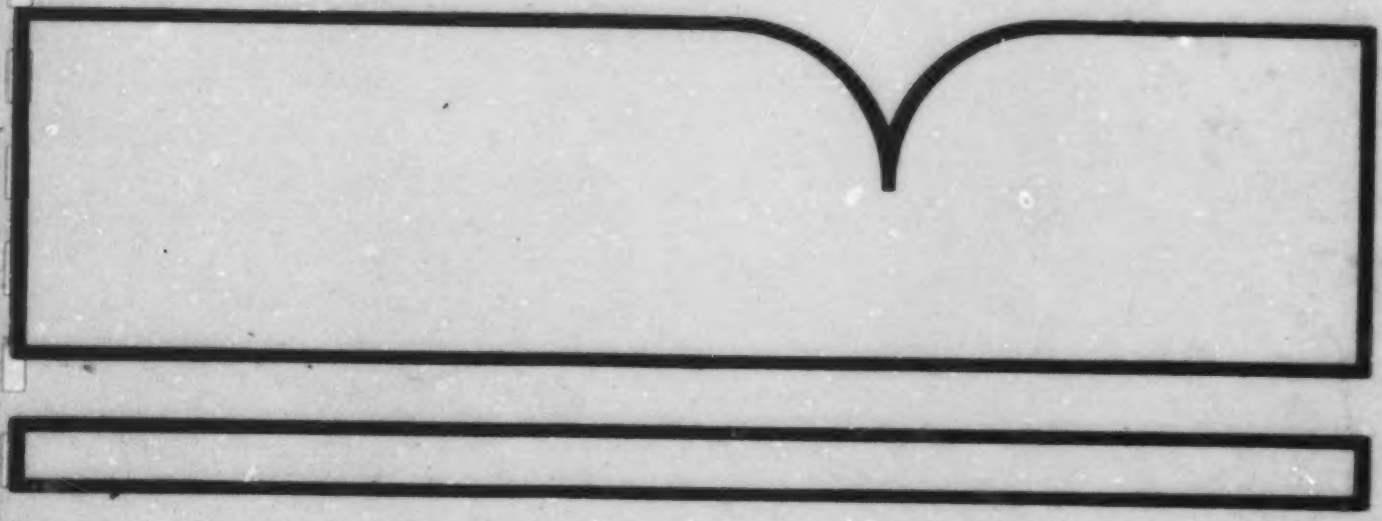

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INDICES IDENTIFYING SUBSURFACE MICROBIAL COMMUNITIES THAT ARE ADAPTED TO ORGANIC POLLUTION

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16. ABSTRACT

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ABSTRACT

The subsurface microbiota and their extracellular excretion products are of primary importance in the maintenance of the ground water resources particularly when the water is polluted. With the development of quantitative biochemical assays for biomass, community structure, and metabolic activities of the microbes of the subsurface sediments, it proved possible to search for assays that correlated with exposure to pollution. The subsurface sediments from an abandoned creosote waste deposit showed that exposure to pollution increased the total microbial biomass as measured by the extractable phospholipid; shifted the community structure by decreasing the proportion of gram positive bacteria that form glycerol teichoic acids and induced distinctive changes in the ester-linked phospholipid fatty acids. Pollution of the subsurface sediments caused a shift in the nutritional status to a more balanced growth condition in the unsaturated zone reflected in an increased rate of phospholipid formation relative to the formation of the endogenous storage polymer beta hydroxy-alkanoate measured after a short exposure to ^{14}C-acetate. In the saturated zone, contamination induced unbalanced growth.

INTRODUCTION

Ground water is an essential resource that must be protected from pollution. Unfortunately the aquifers on which we depend may be contaminated from a multitude of sources. Recently it has been shown that there is a resident microbial community in the subsurface aquifers that can significantly modify the pollutant composition as plumes move through the sediment (Wilson, 1984). With the development of quantitative methods to describe the biomass, community structure, nutritional status, and metabolic activity of the subsurface microbial community and its extracellular polysaccaride polymers (White et al, 1983a; 1984) it was possible to begin the search for indices that could be utilized to define the responses of the subsurface microbial community to pollution.

METHODS

Sampling Sites--Uncontaminated sediments from the surface soils and subsurface sediments (up to 10 m deep) were recovered from unconsolidated loamy clays, sometimes containing small gravel deposits, on the margin of a flood plain of a small river at Lula, Oklahoma. The surface soil was a rich agricultural soil. Contaminated sediments were recovered from a site in Conroe, Texas, on the Gulf coastal plain from an area covered with a poor and highly weathered soil overlying alternating beds of sands and clays of meander belts. A filled lagoon was the source of the organic phenolic wastes from a creosote waste pit that penetrated the meander belt deposits into deeper transmissive sands that overly an impervious clay. Samples were recovered from an uncontaminated control site (well 14), a site with intermediate contamination (well 16), and a heavily contaminated site (well 15) using the aseptic technique developed at the Robert S. Kerr Environmental Research Laboratory (Wilson et al, 1983). The sediments were shipped in ice to the laboratories at Florida State University. The analysis was begun within 24 hours of the sampling. A map of

the sites and the activity of the microbiota in degrading naphthylene, 1-methyl naphthylene, 2-methyl naphthylene, dibenzofuran, fluorene and acenaphthene at this site has been published (Wilson, 1984). The organic contaminants found in the ground water are described elsewhere in this volume (Wang et al, 1984).

The methods utilized in this study are diagrammed in Table 1. References to the detailed procedures are indicated in the Table. The fatty acids are indicated by the number of carbons : number of double bonds with the position of the unsaturation closest to the hydrophobic end (w) indicated by the number. The c or t indicate the cis or trans orientation of the double bonds.

RESULTS AND DISCUSSION

The key to understanding the effects of pollution on the subsurface microbiota were possible with the provision of samples of the subsurface sediments that were uncontaminated by microbes from the surface. The development of a suite of methods to quantitatively assess the biomass, community structure, nutritional status, and metabolic activities of the ground water aquifer microbiota and the extracellular polymers formed by these microbes was then possible. Microbial biomass can be determined by the analysis of cellular components that are universally distributed. The membrane phospholipids are an example. These phospholipids are a part of every cell membrane, and can be quantitatively extracted and assayed with great sensitivity. Their formation parallels cellular growth, they are present in relatively stable amounts in cells growing under subsurface conditions and in surface sediments have a rapid turnover (White et al, 1979; Gehron and White, 1983). The community structure can be monitored by the analysis of cellular components that are restricted to subsets of the total microbial community--the "signatures" of specific groups of microbes (White, 1983). Metabolic activities can be monitored by measuring the rates of turnover or synthesis of biomass or

"signature" components with labeled precursors. Care must be exercised in the methods by which the labeled precursors are added to sediments or artifacts of disturbance can be generated. This is particularly important in surface sediments with complex gradients of reduced carbon and terminal electron acceptors (Findlay and White, 1984a). We have shown that the formation of endogenous storage lipids which form under conditions of unbalanced growth (expansion without multiplication) has proven to be an excellent measure of nutritional status. The formation and turnover of poly beta-hydroxy alkanoate (PHA) for the prokaryotes (Nickels et al, 1979; Findlay and White, 1983) and the triglycerides measured as neutral lipid glycerol for the microeukaryotes (Gehron and White, 1982) have provided quantitative measures of the nutritional status. Measuring the ratio of PHA formation to phospholipid fatty acid formation from ^{14}C-acetate has proved an excellent measure of the disturbance artifact in measurements of metabolic activities in sediments (Findlay and White, 1984a; 1984b).

The subsurface aquifer microbiota form polysaccharide exopolymers in large amounts. These polymers contain uronic acids which on reduction with $NaBD_4$ can be assayed quantitatively by GC/MS (Fazio et al, 1982). The uronic acid components of the polymer can be utilized to define the extracellular localization of these polymers. These polymers tend to be formed in largest amounts under conditions of metabolic stress (Uhlinger and White, 1983).

With these tools in hand it was possible to show that in subsurface clays and sands recovered from 2 to 420 m below the surface from locations in 5 states, a unique subsurface microbial community exists (Gehron and White, 1983; Gehron et al, 1984; White et al, 1983a; 1983b; 1984). This community is sparse--the equivalent of 10^6 to 10^7 bacteria per g dry wt. This is some 10-100 fold less than surface sediments by several biomass measures.

The subsurface sediments contain microbes with a unique complement of

ester-linked phospholipid fatty acids. The profile of fatty acids differs from surface soils, shallow and deep sea sediments and several types of biofilms. The subsurface sedimentary microbiota are characterized by high proportions of short branched and saturated fatty acids characteristic of bacteria. There is an absence of polyenoic fatty acids characteristic of microeukaryotes. There are high levels of plasmalogen phospholipids, cyclopropane fatty acids, and ester-linked phospholipid hydroxy fatty acids characteristic of facultative and obligatory anaerobic prokaryotic microbes. The ratios of total fatty acids to muramic acid, a unique prokaryotic wall component, indicate the community is exclusively prokaryotic, although some protozoal cysts and yeasts have been recovered.

The subsurface microbiota contains a high proportion of gram positive organisms when compared to surface soils. The organisms of the uncontaminated subsurface sediments are nutritionally stressed as evidenced by the high levels of PHA to phospholipid and the large amounts of uronic acid containing extracellular polymers.

The effects of contamination of the subsurface microbiota by phenolic polycyclic organics from the waste pit of an abandoned creosote plant from Conroe, Texas were examined. Contamination significantly increased the microbial biomass as measured by the extractable phospholipid phosphate (Table 2 A). The most contaminated site had the highest biomass. The highest and lowest means are significantly ($P < 0.05$) different by analysis of variance. The presence of contamination shifts the proportions of gram posistive to gram negative bacteria from a gram positive rich microbiota to one progressively more gram negative (Table 2 B). The usual increase in gram positive cells as the depth of the sediment increases (Gehron et al, 1984) is reversed when the subsurface sediments are contaminated. The very low levels of ribitol teichoic

acid (compared to glycerol teichoic acid) seen in the deeper subsurface sediments is preserved in contaminated sediments.

Possibly the most sensitive and quantitative measures of the shifts in microbial community structure come from the analysis of the ester-linked phospholipid fatty acids (White, 1983). A sensitive analysis of these data, in which the specific fatty acids that contribute most to the differences between samples can be derived from a stepwise discriminant analysis of the proportions of the fatty acids (Federle et al, 1983). The results of the discriminant analysis of the logarithms of the ratios of ester-linked phospholipid fatty acids to palmitic acid (16:0) between the control and two contaminated subsurface sites are given in Table 3. The graphical representation of this analysis is given in Figure 1. Discriminant function 1 which accounted for 98.8% of the variance clearly differentiated the three subsurface samples. The total fatty acids, the 18:1w7c (a measure of the activity of the anaerobic desaturase pathway) and a long chain saturated fatty acid 22:0 (possibly characteristic of the more complex bacteria like the actinomycetes), were found in highest proportions in the uncontaminated samples. The fatty acids, 18:2w6 and 18:1w9c, were progressively more concentrated in the samples from contaminated sites. Knowing the proportions of the five fatty acids in a given sample allowed assignment to the correct group with an accuracy of 100%.

Contamination of the subsurface environment could also be detected in the metabolic activity and nutritional status of the microbiota. By exposing a slurry of the sediment samples to sodium acetate-^{14}C for 20 minutes, the rates of formation of phospholipid fatty acids could be compared to the rates of formation of the endogenous storage polymer PHA. Balanced growth is indicated by an increase in the ratio of phospholipid synthesis to PHA synthesis; unbalanced growth is indicated by a decrease in this ratio (Nickels et al, 1979, Findlay and White, 1983; 1984a). Contamination in the saturated zone results in

low metabolic activity and unbalanced growth; contamination in the unsaturated and presumably aerated zone results in high metabolic activity and balanced growth (Table 2 C). The disturbance artifact resulting from the suspension of the sediment in a slurry for a 20 minute exposure did not result in cell division as determined by the incorporation of ^{3}H-thymidine into DNA in subtidal estuarine muddy sediments (Findlay and White, unpublished data).

CONCLUSIONS

Despite the complexities in the sediments from the Conroe, Texas site, it proved possible to define some indices that reflect pollution of the subsurface microbial communities. With increasing exposure to the complex phenolic organics of a creosote waste, the biomass of the microbiota increased. The community structure shifted to a more gram negative microbiota with a distinctive pattern of ester-linked phospholipid fatty acids, and the metabolic activity in the unsaturated zone increased with a shift in nutritional status to a more balanced growth condition. The biotransforming activity of the microbiota of these sediments seems to be largely aerobic (Wilson, 1984), suggesting that the catabolism of the polycyclic pollutants induced the shifts in community structure and the marked increase in metabolic activity. The saturated zone sediments from the contaminated sites showed decreased metabolic activity and a nutritional status characteristic of unbalanced growth.

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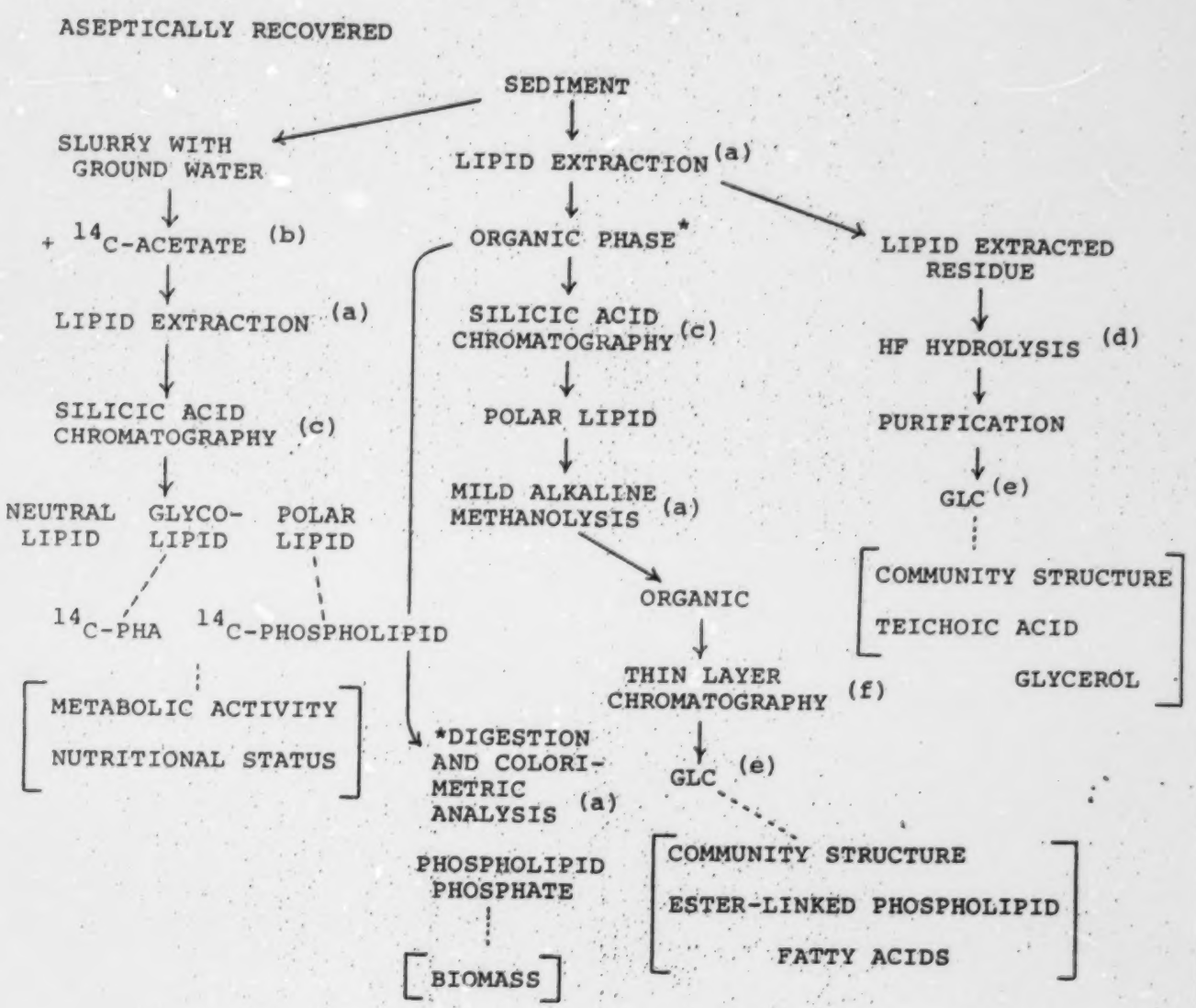
TABLE 1. ANALYSIS OF CONTAMINATED SUBSURFACE SEDIMENTS



(a) White et al., 1979

(b) Findlay and White, 1984a; 1984b

(c) Gehron and White, 1983

(d) Gehron et al., 1984

(e) Gas liquid chromatography

(f) Bobbie and White, 1980

TABLE 2. Effects of contamination of the subsurface sedimentary microbiota on the biomass, community structure, metabolic activity and nutritional status.

	Control	Contaminated	Most Contaminated
Site	14	16	15
A.			
Phospholipid (n moles/g dry wt.)	0.6 (0.5)[a]	1.0 (0.8)	1.9 (0.5)
B.			
Ratio teichoic acid glycerol /phospholipid	56 (56)	20.9 (34)	5.4 (1.0)
C.			
Phospholipid synthesis	3200[b,c]	7800[c]	13000[c]
		176 (120)[d]	152 (80)[d]
PHA synthesis	220[c]	270[c]	590[c]
		103 (92)[d]	130 (10)[d]
Ratio phospholipid/PHA	15[c]	29.9[c]	22[c]
		1.7(1.0)[d]	1.2 (1.2)[d]

[a] Data given as mean (standard deviation).

[b] Total disintegrations per minute per 10 g dry wt sediment after a 20 minute exposure at 25°C in 10 ml well water to 2 μCi sodium acetate-1-^{14}C.

[c] Samples from the unsaturated zone.

[d] Samples from the saturated zone.

TABLE 3. Ratios of ester-linked phospholipid fatty acids/16:0 that contribute to the discrimination of the control, contaminated and most contaminated subsurface aquifer microbial communities.

Discriminating variable[a]	F to Remove[b]	Canonical Discriminant Function Coeficient Z[c]	
Fatty acid		Function 1	Function 2
Total fatty acids	6.8	29	- 7.9
18:2w6	4.6	- 23.8	9.3
18:1w9c	5.4	- 7.4	- 0.2
18:1w7c	2.5	4.1	1.1
22:0	14.2	8.3	- 0.4
% of total variance		98.8	1.2

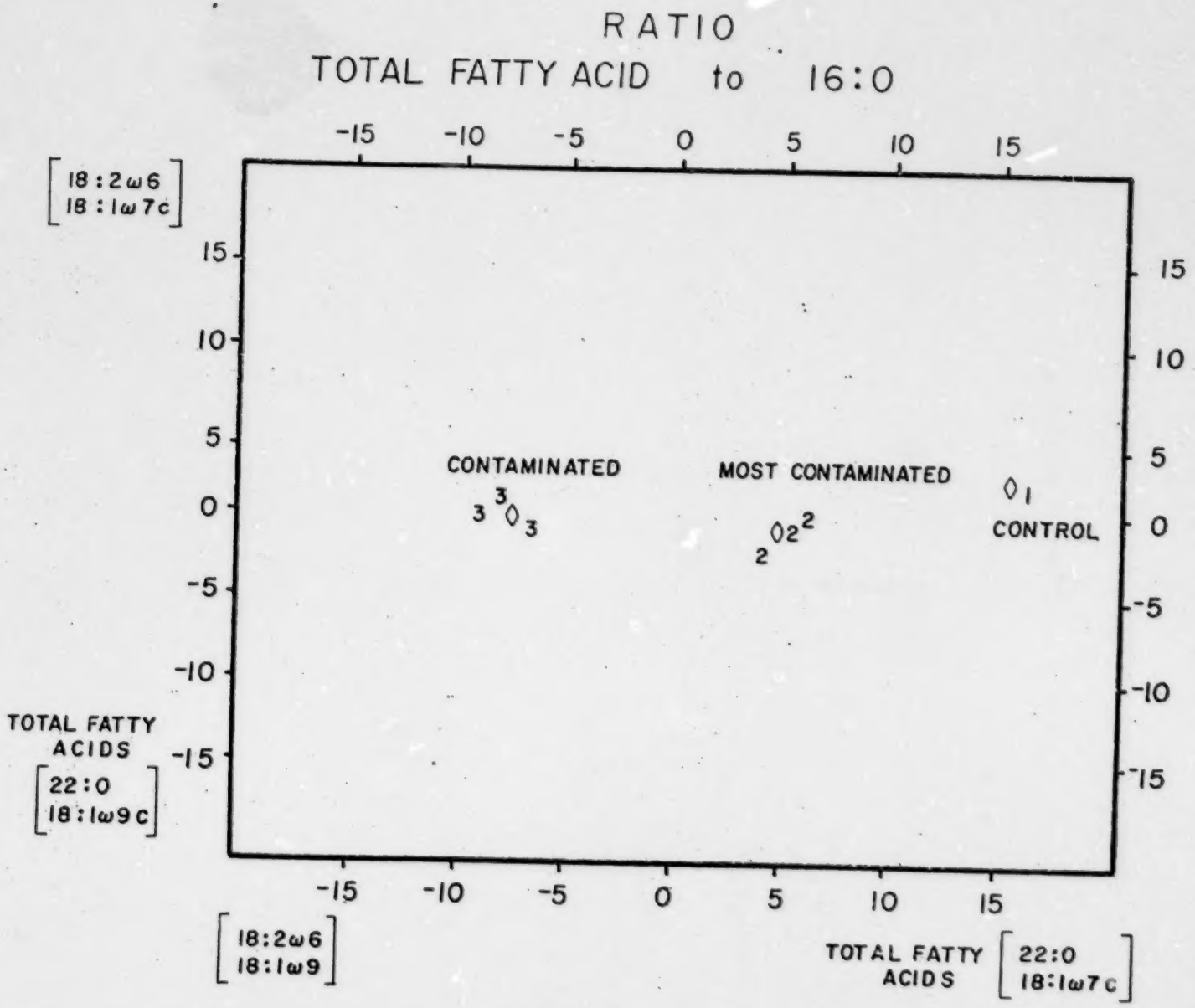
[a] Order of entry into stepwise discriminant analysis. Variables not listed did not contribute to the discrimination.

[b] F to remove indicates the penalty for the removal of the variable from the discriminant function.

[c] Standardized coefficient (Z). Those variables with the largest value contribute most to the discrimination by an individual discriminant function.

FIGURE LEGEND

Figure 1. Discriminant analysis of ratios of fatty acids to palmitic acid from the phospholipids of the microbiota recovered from uncontaminated (1), contaminated (3), and most contaminated (2) subsurface aquifer sediments.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TOTAL FATTY ACID to 16:0
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[18:2ω6 / 18:1ω7c]
15
10
5
0
-5
-10
-15
CONTAMINATED
MOST CONTAMINATED
CONTROL
TOTAL FATTY ACIDS [22:0 / 18:1ω9c]
[18:2ω6 / 18:1ω9]
TOTAL FATTY ACIDS [22:0 / 18:1ω7c]

FIGURE 1